

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising: (a) one or more recombination sites; and (b) one or more topoisomerase recognition sites and/or one or more topoisomerases.

2. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule is a circular molecule.

3. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule comprises two or more recombination sites.

4. The nucleic acid molecule of claim 3, wherein at least one of said two or more recombination sites flanks each end of a topoisomerase recognition site in said molecule.

5. The nucleic acid molecule of claim 1, wherein said recombination sites are selected from the group consisting of:

- (a) *attB* sites,
- (b) *attP* sites,
- (c) *attL* sites,
- (d) *attR* sites,
- (e) *lox* sites,
- (f) *psi* sites,
- (g) *dif* sites,
- (h) *cer* sites,
- (i) *frt* sites,

and mutants, variants, and derivatives of the recombination sites of (a), (b), (c), (d), (e), (f), (g), (h) or (i) which retain the ability to undergo recombination.

6. The nucleic acid molecule of claim 1, wherein said topoisomerase recognition site is recognized and bound by a type I topoisomerase.

7. The nucleic acid molecule of claim 6, wherein said type I topoisomerase is a type IB topoisomerase.

8. The nucleic acid molecule of claim 7, wherein said type IB topoisomerase is selected from the group consisting of eukaryotic nuclear type I topoisomerase and a poxvirus topoisomerase.

9. The nucleic acid molecule of claim 8, wherein said poxvirus topoisomerase is produced by or isolated from a virus selected from the group consisting of vaccinia virus, Shope fibroma virus, ORF virus, fowlpox virus, molluscum contagiosum virus and *Amsacta moorei* entomopoxvirus.

10. A vector comprising the nucleic acid molecule of claim 1.

11. The vector of claim 10, wherein said vector is an expression vector.

12. A vector selected from the group consisting of pcDNAGW-DT(sc), pENTR-DT(sc), pcDNA-DEST41, pENTR/D-TOPO, pENTR/SD/D-TOPO, pcDNA3.2/V5/GWD-TOPO and pcDNA6.2/V5/GWD-TOPO.

13. A host cell comprising the isolated nucleic acid molecule of claim 1.

14. A host cell comprising the vector of claim 10.

15. A host cell comprising the vector of claim 12.

16. An *in vitro* method of cloning a nucleic acid molecule comprising:

- (a) obtaining a first nucleic acid molecule to be cloned;
- (b) mixing said first nucleic acid molecule to be cloned *in vitro* with a second nucleic acid molecule comprising at least a first topoisomerase recognition site flanked by at least a first recombination site, and at least a second topoisomerase recognition site flanked by at least a second recombination site, wherein said first and second recombination sites do not recombine with each other, and at least one topoisomerase; and
- (c) incubating said mixture under conditions such that said first nucleic acid molecule to be cloned is inserted into said second nucleic acid molecule between said first and second topoisomerase recognition sites, thereby producing a first product molecule comprising said first nucleic acid molecule to be cloned between said first and second recombination sites.

17. The method of claim 16, wherein the second nucleic acid molecule is a vector.

18. The method of claim 16, wherein said first nucleic acid molecule to be cloned is a linear nucleic acid molecule.

19. The method of claim 18, wherein said linear nucleic acid molecule is a blunt-end nucleic acid molecule.

20. The method of claim 16, wherein said first nucleic acid molecule to be cloned is a PCR product.

21. The method of claim 16, wherein said first nucleic acid molecule to be cloned comprises at least one open reading frame.

22. The method of claim 16, further comprising contacting said first product molecule with at least one third nucleic acid molecule comprising at least a third and fourth recombination sites that do not recombine with each other, under conditions favoring recombination between said first and third and between said second and fourth recombination sites, thereby producing at least one second product molecule.

23. The method of claim 22, wherein the third nucleic acid molecule is a vector.

24. The method of claim 16, further comprising inserting said first product molecule into a host cell.

25. The method of claim 17, further comprising inserting said first product molecule into a host cell.

26. The method of claim 22, further comprising inserting said second product molecule into a host cell.

27. The method of claim 23, further comprising inserting said second product molecule into a host cell.

28. The method of 17, wherein said vector is an expression vector.

29. The method of 23, wherein said vector is an expression vector.

30. The method of claim 16, wherein said second nucleic acid molecule comprises at least one additional nucleic acid sequence selected from the group consisting of a selectable marker, a cloning site, a restriction site, a promoter, an operator, an operon, an origin of replication, and a gene or partial gene.

31. The method of claim 22, wherein said third nucleic acid molecule comprises at least one additional nucleic acid sequence selected from the group consisting of a selectable marker, a cloning site, a restriction site, a promoter, an operator, an operon, an origin of replication, and a gene or partial gene.

32. The method of claim 16, wherein said first and second recombination sites are selected from the group consisting of:

- (a) *attB* sites,
- (b) *attP* sites,
- (c) *attL* sites,
- (d) *attR* sites,
- (e) *lox* sites,
- (f) *psi* sites,
- (g) *dif* sites,
- (h) *cer* sites,
- (i) *frt* sites,

and mutants, variants, and derivatives of the recombination sites of (a), (b), (c), (d), (e), (f), (g), (h) or (i) which retain the ability to undergo recombination.

33. The method of claim 22, wherein said third and fourth recombination sites are selected from the group consisting of:

- (a) *attB* sites,
- (b) *attP* sites,
- (c) *attL* sites,
- (d) *attR* sites,
- (e) *lox* sites,
- (f) *psi* sites,
- (g) *dif* sites,
- (h) *cer* sites,
- (i) *frt* sites,

and mutants, variants, and derivatives of the recombination sites of (a), (b), (c), (d), (e), (f), (g), (h) or (i) which retain the ability to undergo recombination.

34. The method of claim 32, wherein said *lox* sites are selected from the group consisting of *loxP* sites and *loxP511* sites.

35. The method of claim 33, wherein said *lox* sites are selected from the group consisting of *loxP* sites and *loxP511* sites.

36. The method of claim 16, wherein said topoisomerase is a type I topoisomerase.

37. The nucleic acid molecule of claim 36, wherein said type I topoisomerase is a type IB topoisomerase.

38. The nucleic acid molecule of claim 37, wherein said type IB topoisomerase is selected from the group consisting of eukaryotic nuclear type I topoisomerase and a poxvirus topoisomerase.

39. The nucleic acid molecule of claim 38, wherein said poxvirus topoisomerase is produced by or isolated from a virus selected from the group consisting of vaccinia virus, Shope fibroma virus, ORF virus, fowlpox virus, molluscum contagiosum virus and *Amsacta moorei* entomopoxvirus.

40. The method of claim 22, wherein said product nucleic acid molecule and said third nucleic acid molecule are combined in the presence of at least one recombination protein.

41. The method of claim 40, wherein said recombination protein is selected from the group consisting of:

- (a) Cre;
- (b) Int;
- (c) IHF;
- (d) Xis;
- (e) Fis;
- (f) Hin;
- (g) Gin;
- (h) Cin;
- (i) Tn3 resolvase;
- (j) TndX;
- (k) XerC; and
- (l) XerD.

42. The method of claim 40, wherein said recombination protein is Cre.

43. The method of claim 40, wherein said recombination protein is selected from the group consisting of Int, Xis, IHF and Fis.

44. A kit comprising the isolated nucleic acid molecule of claim 1.

45. The kit of claim 44, further comprising one or more components selected from the group consisting of one or more topoisomerases, one or more recombination proteins, one or more vectors, one or more polypeptides having polymerase activity, and one or more host cells.

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